

A STUDY OF PATHOGENIC TORULAE

BY

DORIS SCHNEIDER COOK

A.B., University of Illinois, 1932

M.S., University of Illinois, 1933

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN
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OF THE UNIVERSITY OF ILLINOIS,

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A STUDY OF PATHOGENIC TORULAE

The study of relation of molds, yeasts, and yeast-like fungi to disease dates back to the seventeenth century, when Hooke described a higher fungus causing disease of leaves of the damask rose. With increasing interest in pathogenic bacteria, fungus diseases were studied to a lesser extent. Recently, however, renewed interest in pathogenic fungi is evidenced by frequent reports in medical literature of cases of mycosis, blastomycosis, aspergillosis, cryptococcosis, and torulosis.

The causative agents in torulosis are yeast-like fungi which do not form ascospores, but reproduce only by budding both in tissues and in cultures. Infection produced in certain tissues is characterized by cell lysis rather than by inflammation, hence the name *Torula histolytica*. Reports in the literature of torulosis in man probably do not indicate the true incidence of infection. Many cases are not recognized until autopsy following antemortem diagnoses of pulmonary tuberculosis, pneumonia, meningitis, encephalitis, brain tumor or abscess, senile dementia, and paresis; probably many more cases are never recognized.

Information on *Torula* infection is complicated 1) by multiplicity of names under which torulosis has been described, i.e., blastomycosis, cryptococcosis, dermatomycosis, granulomatosis, and chromoblastomycosis; and 2) by the diversity of generic names for the causative organism, i.e., *Torula*, *Torulopsis*, *Rhodotorula*, *Mycotorula*, *Eutorula*, *Cryptococcus*, and *Blastomycoides*. The most commonly used specific names are *Torula histolytica* Stoddard and Cutler, 1916, and *Cryptococcus hominis* (Busse) Vuillemin, 1901. The two diseases most easily confused are American blastomycosis, early described by Gilchrist, and true *Torula* infections also called European blastomycosis, described by Busse and Buschke. Torulosis has been characterized as follows: an insidious infection in which are found encapsulated round or oval, budding, thick-walled yeast-like cells, usually 2-8 μ in diameter, which never form spores or mycelium even on culture; the colonies of which are cream-colored, moist, smooth, and mucoid, and change to yellow or brown as they grow older. Central nervous system is involved chiefly, lungs, viscera, and skin to a lesser extent. Pathology is characterized by gelatinous tumors with slight inflammatory reactions, marked endothelial hyperplasia, foreign body giant cells, cysts in brain and occasional caseation, and in certain tissues, lysis rather than inflammation.

The present confusion in classification and nomenclature of pathogenic yeast-like fungi probably cannot be corrected until more is known about these organisms. In this investigation morphological, biochemical, and serological tests were carried out for the purpose of comparing characteristics of 114 strains of asporogenous yeasts isolated mainly from pathological conditions with the hope that some estimate of their relationship might be determined. Results here reported, obtained by using the same methods and media under identical conditions, are intended to contribute to knowledge of these yeast-like fungi which have generally been classified in the genera *Torula*, *Cryptococcus*, and *Torulopsis*. Such studies are necessary for recognition of the various species and for classification.

Strains studied were obtained from the American Type Culture Collection, University of Illinois, and from various other collections and private sources as new cases were reported in the literature. Strains are listed by names which cultures bore when received for the collection, and undoubtedly represent considerable duplication of species. Information regarding source and history of cultures was not always available.

Morphology was determined macroscopically by observing appearance and consistency of growth on dextrose agar slants and of giant colonies on wort and dextrose agar; and microscopically by measuring 24-hour cells with an ocular micrometer. Certain giant colonies were viable at the end of 28 months.

Ability to ferment carbohydrates has been considered important for classification of yeasts and yeast-like fungi. Nutrient broth containing 0.25 to 1% carbohydrate and .0025% brom cresol purple in ordinary culture tubes was inoculated with each strain and observed for production of acid and gas daily for 10 days, then at intervals up to at least 14 days. Considerable variation in speed and amount of fermentation of 18 carbohydrates was noted.

Action on litmus milk was determined at the end of 24 to 48 hours and one month. The milk test, as used by Stovall (1932) and later by Fisher and Arnold (1936) as one criterion in classification, enabled us to determine ability of the strains to coagulate skim milk.

Since these organisms grow poorly on plain gelatin, dextrose was added and cultures incubated at 20°C. at least one month to determine ability to liquefy gelatin.

Of the 29 strains which indicated some reduction of nitrate in dextrose nitrate broth on adding sulfanilic acid and dimethyl-

alphanaphthyl amine, 16 produced very slight or almost negligible coloration.

The following tests were employed to obtain some indication of pathogenicity: hemolysis, skin tests, animal inoculation, and agglutination. Since lysis has been described as characteristic of brain lesions produced by *Torula histolytica*, dextrose blood agar plates were used in an attempt to demonstrate this lytic action on cells (horse red blood cells) *in vitro*. Although 90 out of 109 strains altered the medium in some way, reactions were difficult to read, and were far less satisfactory than those secured with streptococci. However, hemolysis was estimated as alpha or beta.

Intradermal inoculations were made on rabbits according to a method used by Fisher and Arnold (1936) but modified by increasing duration of observation until all signs of reaction had disappeared from the site of inoculation. Forty-one of 111 strains produced sufficient skin reaction to indicate pathogenicity; 40 strains produced a slight reaction; and 30 had no visible effect.

Healthy albino, black and dilute-black hooded, and hairless rats were injected intraperitoneally with 60 strains, including all those which had produced skin reactions in rabbits. After at least one month when the animals were autopsied, 11 strains were recovered, of which two had produced macroscopic visceral lesions.

Attempt was made to secure rabbit sera immune for strains representative of 1) *Torula histolytica*, (2571), 2) *Cryptococcus hominis*, (1465), and 3) pink (red) *Torulae*, (2535). Immune serum (titer 1:320) was obtained only from strain 2535, *Torula* (red) Alvarez, originally isolated from a case of "grassy tongue." Many of the pink or red *Torulae* and a few white and cream colored strains were agglutinated by this serum; chromogenic strains agglutinated in dilution of 1:40 or above are considered to show relationship.

On the basis of the above tests it is possible, tentatively, to separate the strains into rough groups. Of all the strains labeled *Torula histolytica* or *Cryptococcus hominis*, or otherwise labeled and known to be from pathologic sources, 16 strains are considered closely related when strain number 2571 is arbitrarily used as the type. Three strains are less closely related and two others so listed probably do not belong in this group.

The 10 strains agglutinated in higher dilutions by immune serum prepared with culture number 2535 are closely related to, and possibly identical with, strain number 2535; eleven strains

agglutinated at a lower titer are less closely related. Eight red strains with cultural characteristics similar to those of the type strain were not agglutinated.

Of the remaining strains, 11 are grouped as non-pathogenic lactose-fermenting strains and four as non-pathogenic, non-lactose-fermenting strains. The source of most of these strains, cream and milk, would indicate a saprophytic existence.

Two strains (one originally named *Torula hansen*) are probably identical, and three other strains are closely related.

Morphological and cultural characteristics of two strains listed as *Torula colliculosa* suggest identity; whereas two strains labeled *Torula monosa* are undoubtedly not the same, and one of these, although not a red yeast, was agglutinated in dilutions up to 1:320 by immune serum number 2535.

The position of four strains was undetermined. Considerable variation in results was obtained with *Cryptococcus* strains received from Fisher and Arnold.

The genus *Torulopsis* Berlese emend. Ciferri (1925) is accepted as the valid generic name for the non-chromogenic asporogenous yeast-like organisms; and the genus *Rhodotorula*, Harrison (1927), is accepted tentatively for the chromogenic or "red" strains. *Torula glutinis*, the oldest specific name, changed to conform to this nomenclature, would be *Rhodotorula glutinis*. However, no attempt is made to suggest specific names; the aim is rather to indicate a relationship among the many strains from different sources which were available for study. It is not within the scope of this paper to propose a new classification nor to accept any one previously proposed, although the classification of Lodder (1934) is recommended as the best at the present time.

VITA

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